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13. ABSTRACT (Maximum 200 words)
Basic research has been conducted on genetic engineering and synthesis of light-activated protein bacteriorhodopsin, and on a number of analogs for use in device application based on development of new photonic materials derived from bacteriorhodopsin.

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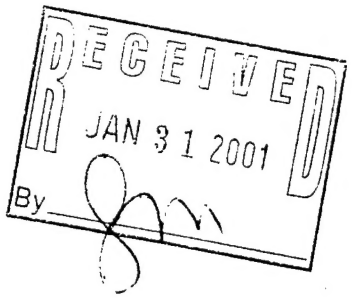

Robert J. Campbell, Ph.D.
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Dear Dr. Campbell:

Enclosed is the final narrative report from Dr. Richard Needleman involving grant DAA H04-96-10093. Please advise if you need any additional information to close out his grant project.

Thank you for your support and approval of the grant extension to October 31, 1999. I have also attached copies of the remaining open invoices awaiting your approval of the final report.

Sincerely,

Daniel Graf
Sponsored Program Administration
313 577-3735

DG:bw

CC R. Needleman
E. Williams, ONR, Chicago
M. Erno

Final Report

Foreword: As the Publication list attests, there are a large number of scientific papers that have resulted from this grant. It would be difficult--and probably not very useful--to summarize the results of the last three years. In addition, detailed summaries in layman's language are contained in the interim reports and need not be repeated here. Instead of concentrating on the diverse scientific questions we have at least partially elucidated, I wish to concentrate here on the practical results of this work--the development of a novel and greatly improved holographic materials based upon bacteriorhodopsin.

Statement of problem studied: Our major goal has been to develop new photonic materials throgh rational synthesis of the light-activated protein bacteriorhodopsin. Bacteriorhodopsin is a amazingly stable material. It is stable and fully functional in acid and base (pH1-12) and at temperatures up to 110 °C. We have made thick dried films of bacteriorhodopsin and used them to construct a real time holographic device which is presently in use at Wright Patterson Air Force base for the measurement of concentration gradients in a wind tunnel. The material serves as a holographic film of great resolution and does not degrade under repetitive laser flashes. In addition, bacteriorhodopsin has been used to build spatial light modulators and artificial vision systems. Although a protein, bacteriorhodopsin can, and has been used in rugged optical and electronic devices.

We developed the first native system for bacteriorhodopsin expression and have used an improved system to make over 300 bacteriorhodopsin mutants including both point and loop insertion mutants (Ni, B., Chang, M., Dusch, A., Lanyi, J. and Needleman, R.B. An efficient system for the synthesis of bacteriorhodopsin in *H. halobium*. Gene, 90:169, 1990).

Currently we use our *E. coli-H. halobium* shuttle vector, pNov^r for expression of bacteriorhodopsin in *H. halobium*. pNov^r contains the entire *H. halobium* plasmid pUBP2, a gene from *H. volcanii* that confers novobiocin resistance (Nov^r), the *E. coli* Tet^r gene, and a polylinker used for cloning *bop* as a *Bam*HI-*Hin*dIII fragment. The mutant *bop* gene is subcloned into pNov^r and transformed into *H. halobium*. Bacteriorhodopsin yields are

comparable to amount of bacteriorhodopsin produced in wild type cells, at about 20 mg/l and but a single purification step is needed to obtain pure protein.

The ease of changing protein structure by site-directed mutagenesis and the inexpensive production of large quantities of pure protein make bacteriorhodopsin an attractive material for device fabrication.

In order to ratioanlly design bacteriorhodopsins with the desired properties--that is, sensitivity and large refractive index shifts--we needed to better understand the amino acid residues which determine the protein's behavior. After an analysis which concentrated on the O state, we developed the material 2357 (D85N/V49A).

Summary of the most important results: As stated in the Foreward, we restrict these here to a discussion of the practical results of this research, in particular the properties of the mutant D85N/V49A (2367).

We have shown hat the mutant D85N/V49A has significantly improved optical properties compared with other forms of blue-membrane bacteriorhodopsin. Absorption studies of the mutant in solution show that it forms the P(490) at light levels comparable to wild-type films. Theoretical calculations based on Kramers-Kronig transformation of light induced absorption data predict that refractive index is three times larger than that of mutant D85N. Holographic measurements performed on gelatin-based films confirm that sensitivity is improved by a factor of 50 over D85N.

The blue form of bacteriorhodopsin is known to have excited state lifetimes ranging from months to years at ambient temperatures. This long lifetime is attractive for applications such as optical data storage or real-time holographic interferometry. The blue membrane is characterized by a ground state absorption peaked in the region of 605 nm and photoexcited states? at 490 nm and 390 nm.[1] The blue form occurs when aspartate-85 is neutral and can therefore be induced in wild-type by low pH or by removing the charged aspartate-85 and replacing it with a neutral residue like asparagine(N). The blue membrane shows significantly reduced optical sensitivity with respect to wild type. That is, the required optical fluence to saturate the transition (bleach or record a hologram in the material) is more than two orders of magnitude

greater than required for wild-type at neutral pH. We have investigated a wide variety of blue membrane mutants to search for one with an improved optical sensitivity. We show that the double mutant D85N/V49A has a sensitivity that is almost two orders of magnitude better than other forms of the blue membrane. In addition several mutants are capable of forming the Q390 state at neutral pH. (

Site-specific and random mutagenesis was used to synthesize a variety of new mutants using D85N as the wild type. Mutants were then grown in small liquid cultures (50ml) and purified by sucrose centrifugation for incorporation into a polyacrylamide gel to an optical density ~1.0 in a 1 cm cuvette. Absorbance spectra were made with Shimadzu UV160U spectrophotometer andwere measured as a function of exposure from a filtered white light source fitted with a 630 nm long pass filter (SRG630). The difference spectra were analyzed to detect mutants with high sensitivityand formation of the Q390 and P480 state under neutral pH conditions. Table 1 summarizes some representative mutants.

Most of the mutants had a ground state around 605 nm and a bleached state in the region of 480 nm. Several mutants including D85A, D85L and the triple D85N/A103C/G213C showed the formation of a state near 390 nm. These mutants offer the possibility of very long lifetimes; however, they were not investigated further as part of this study. Figure 1 shows the greating lifetime

Table 1. Summary of measured and calculated properties for the mutants forming the blue membrane that were investigated in this study.

Mutant	Ground state peak (nm)	ΔOD ground	ΔOD P480	ΔOD Q390	max. Δn at wavelength ($x10^{-4}$)	Relative diffraction efficiency η	Relative bleaching efficiency
D85N	610	27 %	9 %	-	2.4 @ 680 nm	1	1
D85A	622	39 %	-	12 %	3.2 @ 660 nm	1.8	.7
D85E	610	9 %	3 %	-	0.8	0.1	.5

					@ 660 nm		
D85L	588	35 %	18 %	10%	2.7 @ 680 nm	1.3	1.4
D85N/D96N	575	29 %	11 %	-	2.2 @ 660 nm	0.8	1.1
D85N/V49A	610	46 %	12 %	-	4.0 @ 670 nm	2.8	7
D85N/G231C	597	34 %	15 %	-	2.8 @ 650 nm	1.4	-*
D85N/A103C/G231C	600	23 %	7 %	5 %	1.9 @ 680 nm	0.6	-*

* not measured

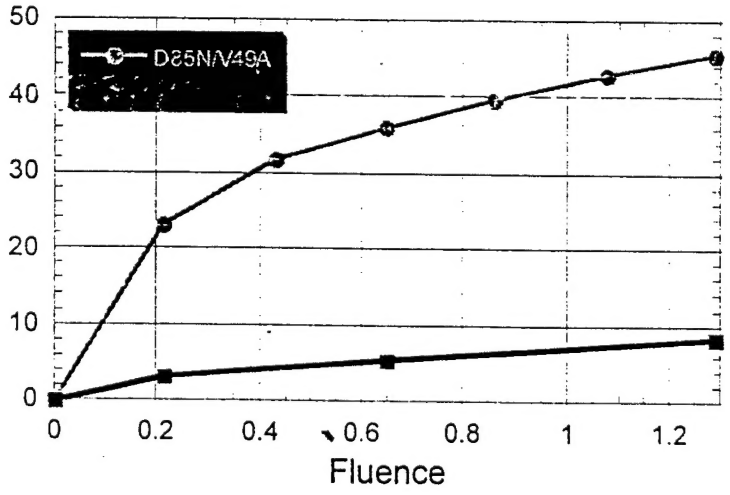

Figure 1. Measured change in absorption as a function of red light fluence for two mutants. Initial slope of D85N/V49A shows a factor of 7 increase over D85N.

Figure 1 shows that the mutant has greatly improved sensitivity over the currently 'best' grating material 85N.

Figure 2 below shows the grating lifetime in the mutant film--clearly long enough for memory storage applications.

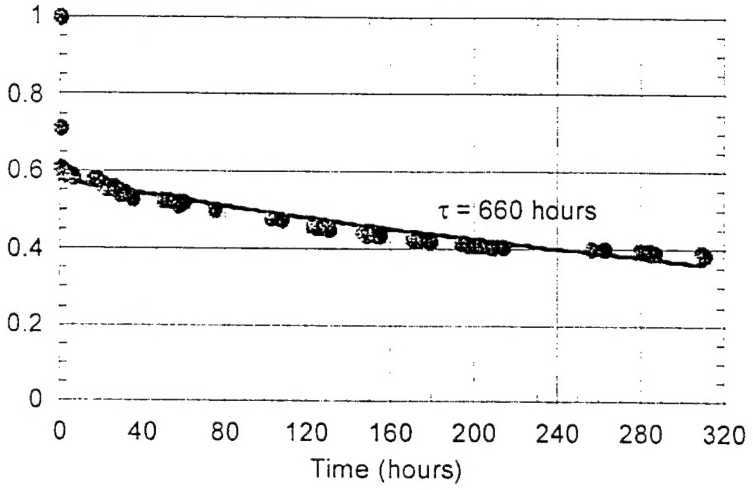

Figure 2. Measured grating lifetime in D85N/V49A film.

The above experiments were done in cooperation with MetroLaser (Irvine CA).

Although the estimated relative diffraction efficiency was a factor of 3 higher for the new mutant, the measured diffraction efficiency was approximately the same for both D85N and D85N/V49A (~1.5%). Small changes in the hydration and pH of the film have been shown to significantly affect the overall diffraction efficiency. Therefore, the variations in the manufacturing of the two films can partially account for this discrepancy. In addition, the non-linear response of the medium when recorded at a large intensity modulation index --reference and object beams close to 1-- results in non-sinusoidal gratings which can also account for the apparent clamping of diffraction efficiency to only a few percent.

We have synthesized and measured the optical absorption properties of several mutants bearing properties similar to the blue membrane. Several mutants were identified that form the so-called "Q390 state" at neutral pH. The double mutant D85N/V49A showed a factor of 50 increase in holographic recording sensitivity compared with the single mutant D85N, and had a similar diffraction efficiency. The increased sensitivity of this mutant and its' long storage lifetime makes this film a good

choice for real-time holographic interferometry systems and optical data storage applications.

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